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Nucleobases

On the Use of Metal Purine Derivatives (M = Ir, Rh) for the Selective Labeling of Nucleosides and Nucleotides

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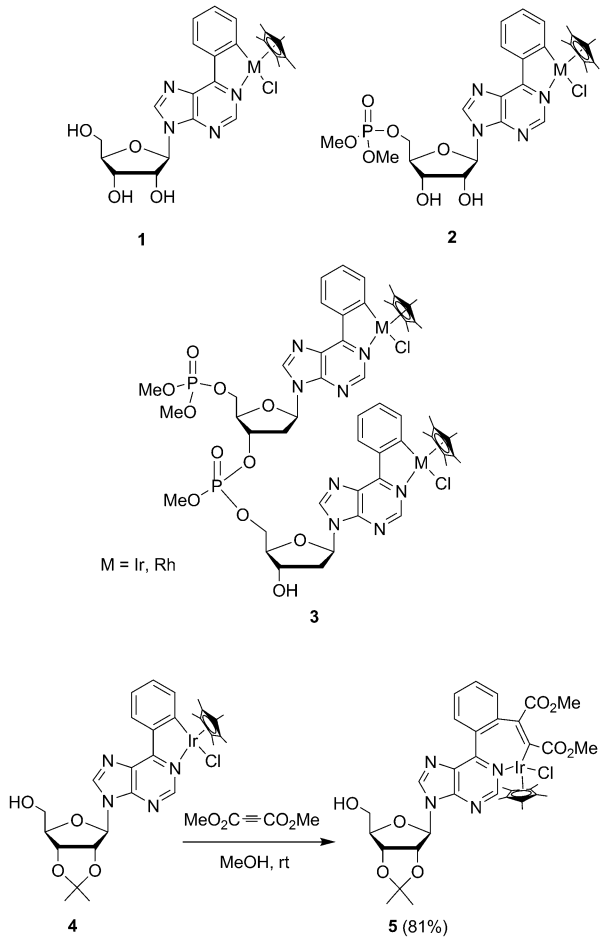
Abstract: The reactions of neutral or cationic Ir^{III} and Rh^{III} derivatives of phenyl purine nucleobases with unsymmetrical alkynes produce new metallacycles in a predictable manner, which allows for the incorporation of either photoactive (anthracene or pyrene) or electroactive (ferrocene) labels in the nucleotide or nucleoside moiety. The reported methodology (metalation of the purine derivative and subsequent marker insertion) could be used for the postfunctionalization and unambiguous labeling of oligonucleotides.

Introduction

The design and synthesis of unnatural nucleic acids is a fascinating area of research.[1] The idea that pairing of purine and pyrimidine derivatives through hydrogen bonding is not an absolute requirement for duplex formation or replication of DNA has opened new ways of approaching the design of bases and base pairs that in many cases have scarce similarity with the natural ones.[1–3] These new bases are not just valuable to be used as part of an expanded genetic alphabet,[4,5] or as the origin of novel interactions in RNA translation,[6] but are also suitable places to introduce specific labeling points, which would occur in applications ranging from fluorescent probes to the design of new nanomaterials.[7,8] Additionally, unnatural nucleic acid bases could also be interesting for their biological activity.[9]

We have reported a general approach to metal arylpurine nucleosides **1**, nucleotides **2**, and dinucleotides **3** having M–C bonds (M = Ir, Rh; Scheme 1).[10] These compounds are susceptible to selective postfunctionalization by means of alkyne insertion reactions. Our preliminary work with iridanucleoside **4** and dimethylacetylene dicarboxylate showed the clean and complete incorporation of a single alkyne unit to form cyclometalated complex **5** in excellent yield (Scheme 1). This simple experiment opens doors to the possibility of postfunctionalization of metalated nucleic acid derivatives in a complete site-selective fashion (the site selectivity will be provided by the initial metal complex). We report herein the regioselective postfunctionalization of metallanucleosides and metallanucleotides



1 2 3 4 **5** (81%)

Scheme 1. Some representative examples of metal (M–C) purine derivatives.

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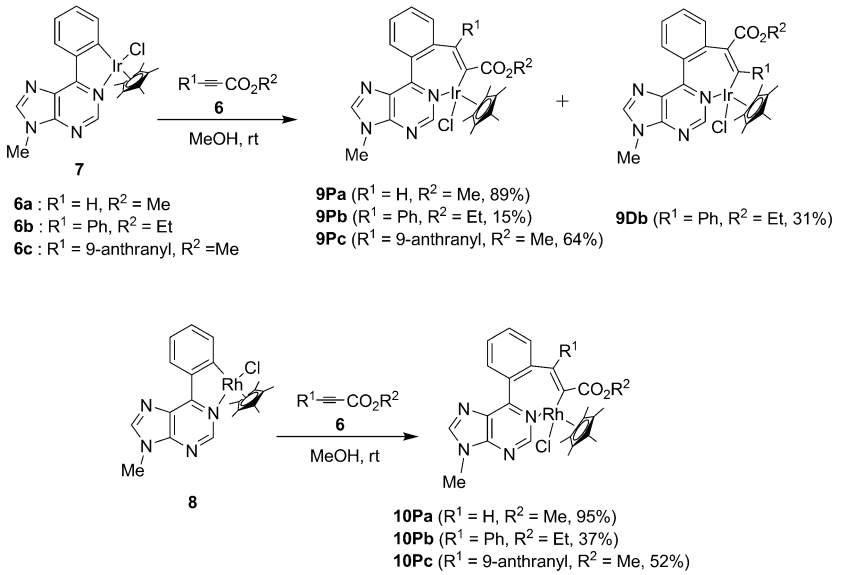
*Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201304091, and contains general procedures, full synthetic details, spectroscopic data, electrochemical studies, and crystallographic data of compounds **9Db**, **10Pc**, **25**, and **28**.*

and explore it as tool to incorporate fluorescent or redox labels in their structures. The successful application of the reported methodology to the selective postfunctionalization of oligonucleotides (metalation of specific modified bases within the chain and subsequent incorporation of the label) could be considered an alternative approach[11] to the incorporation of labeling points in modified DNA fragments.

Results and Discussion

The possibility of effecting insertion reactions on nucleotides with unsymmetrically substituted alkynes **6** was initially tested on cyclometalated *N*-9-methyl-6-phenylpurines **7** and **8** as the model compounds (Scheme 2). Compounds **7** and **8** were prepared in 72 and 69% yields, respectively, from *N*-9-methyl-6-phenylpurine, $[MCl_2Cp^*]_2$ (M = Ir, Rh; Cp* = pentamethylcyclopentadienyl), and AcONa in CH_2Cl_2 at room temperature.[12] The reactions with alkynes **6** were carried out in MeOH at room temperature and the products were isolated by chromatography on silica gel.[13] As two orientations were in principle possible in the insertion process, throughout the discussion we will define P (proximal) as the regioisomer in which the carboxylate group is placed next to the metal fragment in the final product, and D (distal) as that in which the carboxylate is placed next to the 6-phenyl ring (Scheme 2).



Scheme 2. Insertion reactions of model purine derivatives with unsymmetrical alkynes.

Terminal alkynes were tested first against the insertion reaction. The reaction of phenylacetylene with **7** and **8** only led to the recovery of unreacted starting material, together with decomposition byproducts. However, reaction of methyl propiolate **6a** with cyclometalated Ir and Rh complexes **7** and **8** led respectively to the monoinsertion products **9Pa** and **10Pa** bearing the carboxylate group next to the metal fragment, with complete regioselectivity and in excellent yields of isolated products (Scheme 2). The structure and regiochemistry of the reaction products were unequivocally established by mono- and bidimensional spectroscopic analysis, and mass spectrometry.[13]

To determine the influence of steric effects on the regioselectivity of the insertion reaction, ethyl phenyl propiolate **6b** was tested next. Reaction of iridium metallacycle **7** with **6b** led to a mixture of regioisomers **9Pb** and **9Db** (1:1 proximal/distal ratio in the ^1H NMR spectrum of the crude product). The products were separated by chromatography on silica gel and their structures established on spectroscopic and analytical grounds. Additional structural confirmation was obtained by X-ray diffraction analysis of a suitable crystal of complex **9Db** grown by slow diffusion in 1,2-dichloroethane/hexane (Figure 1). In the case of the reaction of alkyne **6b** with rhodium complex **8**, the analysis of the NMR spectrum of the reaction crude showed extensive decomposition and only the signals of the proximal regioisomer **10Pb** could be clearly observed. The product was isolated in 37% yield.

With these results in hand, the reaction of metallacycles **7** and **8** with alkyne **6c** having the bulky 9-anthranyl group was assayed, and led exclusively to the formation of the proximal regioisomers **9Pc** and **10Pc** in good yields of isolated products. The structure and regiochemistry of compounds **9Pc** and **10Pc** were established by analytical and spectroscopic techniques and, for rhodium complex **10Pc**, confirmed by X-ray diffraction analysis of a suitable crystal obtained by slow diffusion

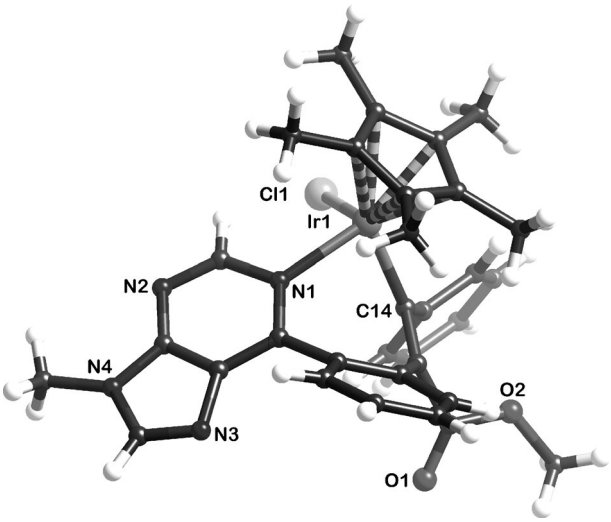


Figure 1. X-ray molecular diagram of complex **9Db**. Selected bond lengths [Å] and angles [°]: Ir–Cl(1) 2.415(2), Ir–N(1) 2.153(5), Ir–C(14) 2.153(5); Cl(1)-Ir-N(1) 88.5(1), C(14)-Ir-N(1) 84.8(2), C(14)-Ir-(Cl) 89.6(2). CCDC-952794 (**9Db**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

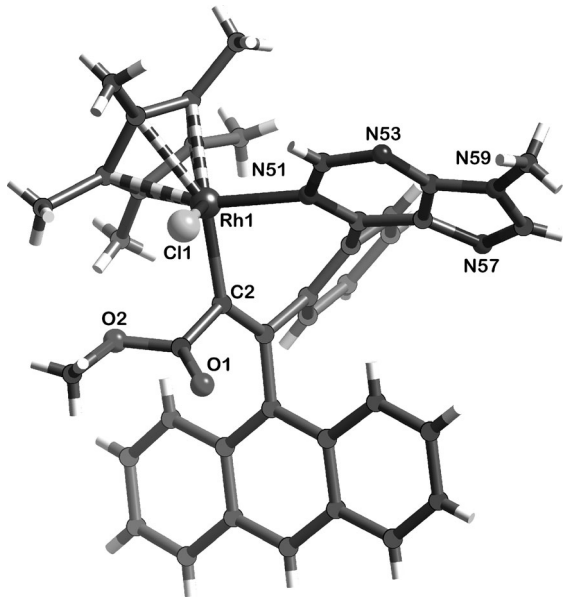
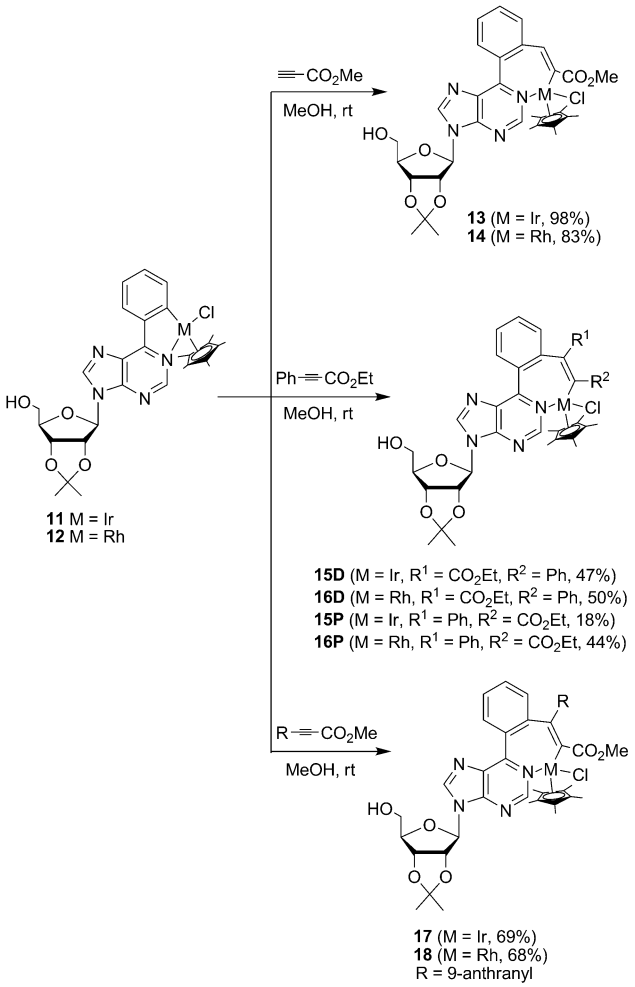


Figure 2. X-ray molecular diagram of complex **10Pc**. Selected bond lengths [Å] and angles [°]: Rh(1)–Cl(1) 2.415(2), Rh(1)–N(51) 2.134(5), Rh(1)–C(2) 2.076(6); Cl(1)-Rh(1)-N(51) 88.9(1), C(2)-Rh(1)-N(51) 87.2(2), C(2)-Rh(1)-Cl(1) 87.9(2). CCDC-952795 (**10Pc**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

in 1,2-dichloroethane/hexane (Figure 2). In both cases, the presence of the 9-anthranyl group exerts a noticeable influence on the signals of the neighboring carboxylate group in their NMR spectra. For example, the OCH_3 protons of **10Pc** appear in the ^{1}H NMR spectrum (25 °C) as two broad signals ($\delta = 3.12$ ppm, 2H, and 2.52 ppm, 1H) that coalesced to a single one at $\delta = 3.09$ ppm at 70 °C in [D_6]DMSO. Additionally, the signal of the CO ester group is hardly visible in the ^{13}C NMR spectrum, in contrast to the strong IR absorption band observed at 1693 cm^{-1}.[14]

It is remarkable how the insertion of propiolates **6a** and **6c** that have substituents so different in size ($R^1 = H$ in **6a** to $R^1 =$ 9-anthranyl in **6c**) takes place with total selectivity, leading in both cases to the proximal regioisomers. Steric and electronic effects,[15] among others,[16,17] have been claimed to determine the regioselectivity of unsymmetrical alkyne insertions in metallacycles. Although the data reported suggest that the bulkier the substituent in the alkyne the higher the preference to place it next to the metal fragment,[15a] it is also true that this conclusion has been drawn from studies just limited to insertions of ethyl phenyl propiolate and ethyl 4,4,4-trifluoro-2-butynoate, with Ph and CF_3 as the bulky groups. None of these reactions was totally regioselective. This behavior is opposite to that reported here for compounds **9Pc** and **10Pc**, which, in addition, are fully regioselective processes. It is our feeling that the control of the regioselectivity in this type of insertion is a more complex matter than the evaluation of electronic/steric effects of the substituents in the alkyne, and would require an in-depth evaluation of the different steps of the process (kinetic and thermodynamic effects).

The excellent results obtained in the alkyne insertions using model purine metallacycles **7** and **8** prompted us to test the reactivity and regioselectivity of the process in more complex metallanucleosides and metallanucleotides **11** and **12** (1:1 diastereomeric mixtures; Scheme 3).[10] In this manner, insertion of



Scheme 3. Insertion of alkynes in arylpurine nucleosides.

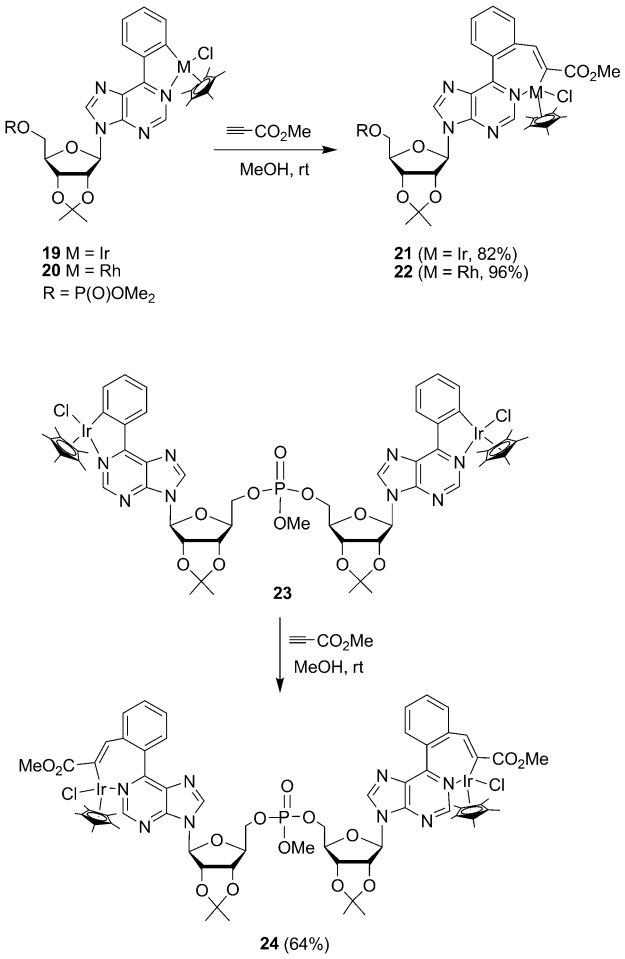
alkynes **6a–c** under the above conditions led in all cases to the expected monoinsertion products, with excellent yields and regioselectivities identical to those found in the model reaction (see above). Reactions with alkynes **6a** and **6c** produced exclusively the proximal regioisomers **13**, **14**, **17**, and **18**, whereas alkyne **6b** led to proximal/distal (1:1) regioisomeric mixtures **15P/15D** for the iridium metallacycle **11** and (7:3) **16P/16D** for the rhodium metallacycle **12** (^{1}H NMR ratios in the crude reaction). All the insertion products were purified by chromatography on silica gel and their structures established by spectroscopic and analytical data.

Insertion reactions of alkyne **6a** were next attempted with metallanucleotides **19** and **20** (1:1 diastereomeric mixtures),[10] which afforded the expected insertion products **21** and **22** with total regioselectivity and in nearly quantitative yields. Fi-

nally, the methodology was assayed with dinucleotide **23**, which formed the bis-insertion iridametallacycle **24** in 64% yield of isolated product (Scheme 4). These results demonstrate the compatibility of the reaction with the phosphate bond.

Due to their photophysical and photochemical properties, anthracene-derived compounds have found diverse applications as fluorescent probes.[18] In the context of our research interest in the postfunctionalization of metalla-DNA fragments, the good yields and total selectivities observed in the insertion of bulky alkyne **6c** (R^1 = 9-anthranyl) in both Ir and Rh metallacycles **7** and **8** let us consider applying this reaction as a regioselective method to incorporate other fluorescent or redox probes in a metallanucleotide. Unfortunately, attempts to achieve the reaction of model purine metallacycles **7** and **8** with alkynes **6d** (R^1 = 1-pyrenyl) and **6e** (R^1 = ferrocenyl) under the conditions shown in Scheme 2 were fruitless, leading to the recovery of unreacted reagents. Heating at reflux in MeOH overnight or carrying out the reaction in a sealed tube did not lead either to the insertion products. It is known that alkyne insertions into cyclometalated complexes are facilitated by substituting the chloride in the starting compounds with acetonitrile to provide cationic complexes.[19] In this regard we prepared cationic MeCN complexes **25** and **26** in excellent yields by stirring metallacycles **7** and **8** with $NaPF_6$ overnight at room temperature (Scheme 5). The structure of the cationic complexes was established by spectroscopic and analytical data and for Ir^{III} complex **25** by X-ray diffraction analysis (Figure 3).

The efficiency of the insertions of terminal alkynes into the model purine cationic complexes **25** and **26** was tested first. Stoichiometric amounts of **25** and phenylacetylene (an alkyne that proved to be unreactive towards model complexes **7** and **8** in the preliminary assays) yielded monoinsertion product **27** as a single regioisomer, in 90% yield of isolated product.[20,21] Excess phenylacetylene cleanly afforded the double insertion product **28**, the structure of which was established by spectroscopic and analytical data and by X-ray diffraction analysis of a suitable crystal obtained by slow diffusion in ether (Figure 3). The structure shows a six-membered metallacycle formed by insertion of two molecules of PhC≡CH into the M–C bond.[22] The organic fragment is bonded through the purine nitrogen atom N(1) and has an asymmetrical η^3 interaction with three of the alkyne carbon atoms C(1), C(14), and C(21). The Ir–C(21) has the shortest bond length (2.108(8) Å) and the Ir–C(14) the longest (2.261(8) Å). The C(21)–



Scheme 4. Insertion of alkynes in arylpurine nucleotides and dinucleotides.

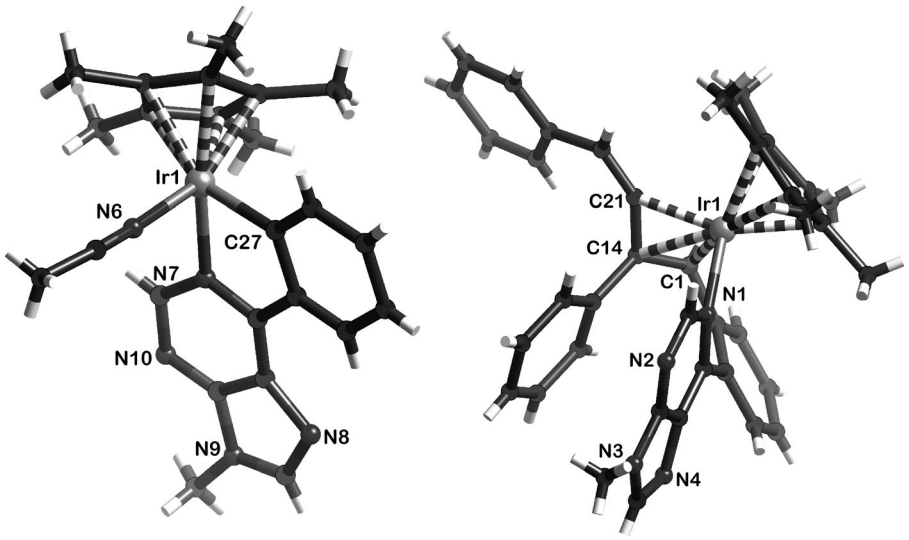
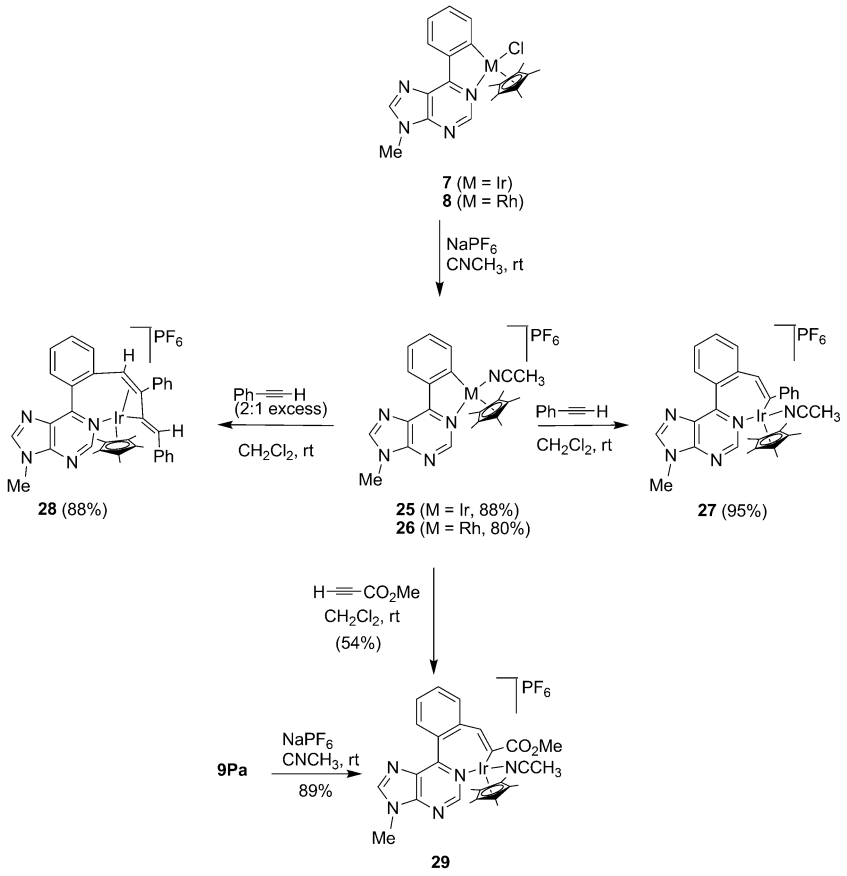


Figure 3. X-ray molecular diagram of compounds **25** (left) and **28** (right). Selected bond lengths [Å] and angles [°]: **25**: Ir(1)–N(6) 2.018(14), Ir(1)–N(7) 2.104(11), Ir(1)–C(27) 1.994(16); N(6)-Ir(1)-N(7) 86.4(4), C(27)-Ir(1)-N(6) 89.0(5), C(27)-Ir(1)-N(7) 77.2(5); **28**: Ir(1)–N(1) 2.118(6), Ir(1)–C(1) 2.156(8), Ir(1)–C(14) 2.261(8), Ir(1)–C(21) 2.108(8), C(1)–C(14) 1.419(11), C(14)–C(21) 1.445(11), C(21)–C(22) 1.303(11); N(1)-Ir(1)-C(1) 87.9(3). CCDC-952794 (**25**), and 952797 (**28**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.



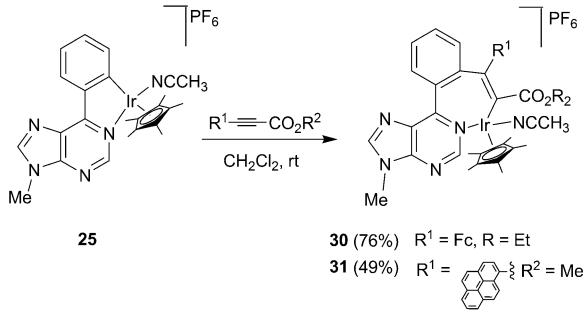
Scheme 5. Reactivity of cationic Ir^{III} arylpurine derivatives with alkynes.

absorption properties of the metallanucleobases **9Pc**, **10Pc, 17, 18**, and **31** relative to those of the corresponding structurally related complexes. UV/Vis spectra of all iridium metallacycles discussed throughout this work are represented in Figures 4–6 and data are collected in Tables 1 and 2.[23] As shown in Figure 4, the insertion of the alkynes **6** in the model neutral metallacycle **7** provokes a dramatic effect in the UV/Vis spectrum, with the almost complete disappearance of the 390 nm absorption band (assignable to metal-to-ligand charge transfer, MLCT) of the starting compound (see the spectra of **9Pa**, **9Pb**, and **9Db**). In this scenario, the incorporation of the 9-anthranyl moiety in the structure of the metallacycle is clearly noticed by the appearance of new bands in the 360–400 nm region (see the spectrum of the anthranyl derivative **9Pc** compared with those of **9Pa** or **9Pb** in Figure 4). A similar discussion could be applied to the UV/Vis spectra in Figure 5A and B and data in Table 2. Thus, relative to parent compound **11**, nucleosides **13** and **15** show the almost complete disappearance of the MLCT band (Figure 5A). The same trend is observed for nucleotides **21** and dinucletotides **24** compared to **19** and **23**, respectively (Figure 5B). Again, the incorporation of the 9-anthranyl moiety in the structure of the metallacycle is shown by the appearance of new absorbances in the 360–400 nm region (see the UV/Vis spectrum of **17** in Figure 5A).

C(22) distance of 1.303(11) Å is typical of a double bond and is shorter than the C(21)–C(14) bond length (1.445(11) Å) within the η^3-allyl group. The phenyl substituent on the central carbon atom of the allyl moiety is placed *anti* with respect to the Cp* on Ir.

Stoichiometric reaction of methyl propiolate **6a** with cationic iridametallacycle **25** led to the expected monoinsertion product **29** as a single proximal regioisomer (54% yield of isolated product; Scheme 5). Monoinserted cationic complexes could also be obtained from the corresponding neutral ones. As an example, **9Pa** was treated with $NaPF_6$ in acetonitrile at room temperature to form **29** in almost quantitative yield.

Once the reaction conditions were tuned, the reactivity of cationic complex **25** towards alkynes **6d** (R^1 = 1-pyrenyl) and **6e** (R^1 = ferrocenyl) was tested. In both cases the insertion products **30** and **31** were obtained in good yields and as single proximal isomers (Scheme 6). Pure products were separated by chromatography on silica gel and their structures established on spectroscopic and analytical grounds.



Scheme 6. Synthesis of cationic Ir^{III} arylpurine derivatives **30** and **31**.

The usefulness of the above methodology when applied to the labeling of unnatural oligonucleotides is related to the existence of a simple technique to efficiently detect the successful incorporation of the label after the reaction. In this regard a study of the UV/Vis spectra was carried out to establish the effect of the presence of anthracene and pyrene labels on the

A similar behavior is observed for the cationic complexes **25**, **29**, and **31**, in which the insertion of the alkyne unit also pro-

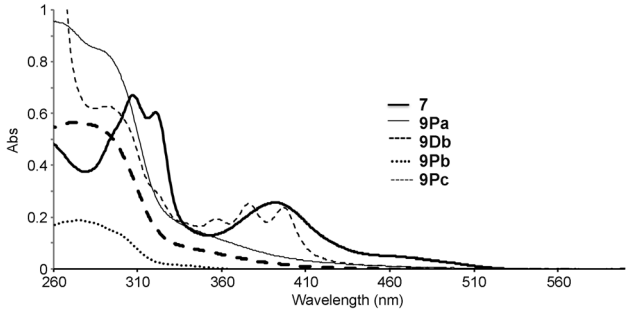


Figure 4. UV/Vis spectra of metallacycles **7** and **9a–c** in CH_2Cl_2 (ca. 10^{-5} M).

Table 1. UV/Vis main absorptions of metallacycles **7** and **9a–c**.

Complex[a]	λ [nm]	$10^{-3}\ \varepsilon$ [$M^{-1}cm^{-1}$]
7	392, 320, 307	8, 19, 21
9Pa	293, 268	26, 30
9Pb	273	5
9Db	274	18
9Pc	396, 376, 356, 293	7, 8, 6, 20

[a] In CH_2Cl_2 (ca. 10^{-5} M).

Table 2. UV/Vis main absorptions of Ir^{III} nucleosides, nucleotides and cationic arylpurines.

Complex[a]	λ [nm]	$10^{-3}\ \varepsilon$ [$M^{-1}cm^{-1}$]
11	400, 321, 307	6, 18, 19
13	292, 268	18, 19
15D	273	18
15P	279	21
17	396, 375, 356, 339	11, 12, 8, 6, 20
19	396, 320, 307	8, 20, 23
21	291, 264	18, 20
23	394, 320, 307	14, 35, 40
24	291, 266	32, 35
25	350, 318, 306	11, 22, 19
29	442, 340	2, 6
31	354, 342, 280	27, 24, 41

[a] In CH_2Cl_2 (ca. 10^{-5} M).

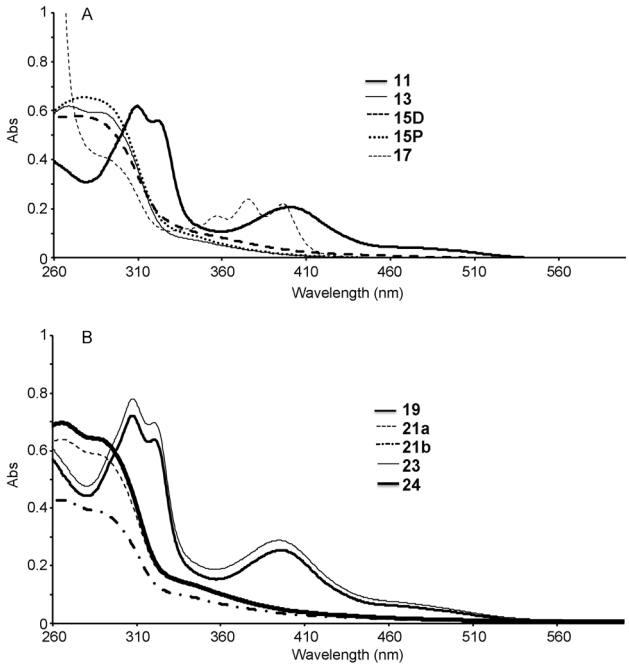


Figure 5. A) UV/Vis spectra of Ir^{III} nucleosides **11**, **13**, **15**, and **17**. B) UV/Vis spectra of Ir^{III} nucleotides **19** and **21** and dinucleotides **23** and **24**. Spectra registered in CH_2Cl_2 (ca. 10^{-5} M).

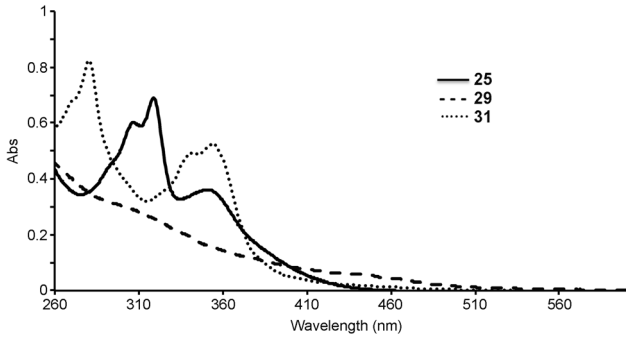


Figure 6. UV/Vis spectra of metallacycles **25**, **29**, and **31** in CH_2Cl_2 (ca. 10^{-5} M).

duces a dramatic decrease of all the absorption bands (see, for example, the UV/Vis spectra of **25** and **29** in Figure 6, Table 2). Now, the incorporation of the pyrenyl group in the structure can be easily detected by the appearance of three new intense bands at 280, 342, and 354 nm (see the spectrum of **29** relative to the pyrenyl derivative **31** in Figure 6, Table 2).

Alternatively, the easiest way to determine the effect of the incorporation of a redox label in the prepared metallacycles is the study of their redox properties. In this regard, the electrochemistry of the neutral and cationic cyclometalated products made was carried out. Full data and cyclic voltammograms are shown in the Supporting Information. All iridium complexes studied show two different redox events. The first one is a reversible oxidation at potentials between 0.9 and 1.3 V (assignable to a metal-centered electron-transfer process) and in the range of Ir^{III}/Ir^{IV} reported values.[24] The second step, at oxidation potentials between 1.2 and 1.7 V, is irreversible and may be attributable to a metal-centered Ir^{IV}/Ir^{V} process or to a ligand-centered process. The model rhodium complexes studied exhibit similar redox behavior but, as expected, the oxidation takes place at higher potentials than for the iridium counterparts. Also, the alkyne insertion products generally show oxidation waves at higher potential values than the cyclometalated starting materials.

The effect of the incorporation of a ferrocenyl substituent was evaluated by studying the redox properties of cationic complex **30** (Figure 7, Table 3). Model cationic complex **25** shows an oxidation wave at 1.59 V, hardly visible in the product of insertion of methyl propiolate **29**. The incorporation of the ferrocenyl moiety in **30** is clearly detectable by the appearance of a reversible oxidation wave assignable to the ferrocene/ferrocenium couple (Fc/Fc^+; $E_{1/2} = 0.47$ V) together with a new quasireversible wave at ($E_{1/2} = 0.89$ V), very likely due to

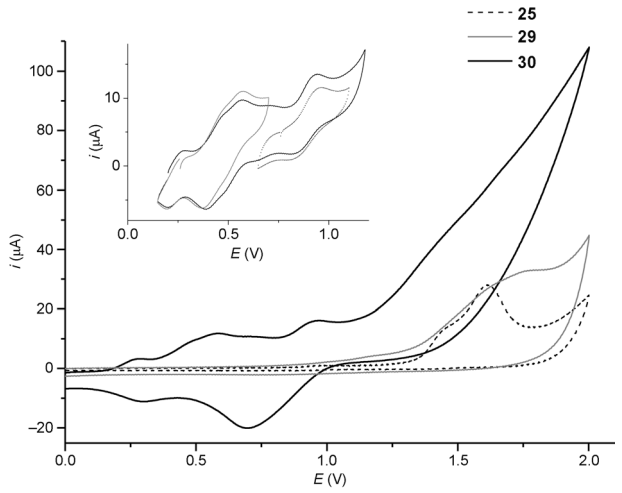


Figure 7. Cyclic voltammetry of compounds **25**, **29**, and **30** in CH_2Cl_2 (ca. 10^{-3} M) with Ag/AgCl (3 M) as reference.

Table 3. Electrochemical data for ferrocenyl complexes, **25**, **29** and **30** (in CH_2Cl_2, with 3 M Ag/AgCl as reference).[a]

Complex	$E_{pa}1$	$E_{pa}2$	$E_{1/2}$	$E_{pa}2$	$E_{pc}2$	$E_{1/2}$
25	1.59	–	–	–	–	–
29	1.64	–	–	–	–	–
30	0.57	0.38	0.47	0.95	0.84	0.89

[a] Values are given in volts.

the oxidation of the Ir^{III}. The presence of the ferrocenyl group shifts the oxidation potential of the metal to lower values relative to the model cationic complex **25**. This behavior is similar to that of the neutral complexes, which show a reversible oxidation wave for the metal (see the Supporting Information).

Conclusion

The reactions of neutral or cationic Ir^{III} and Rh^{III} derivatives of phenyl purine nucleobases with unsymmetrical alkynes produce new metallacycles with high proximal regioselectivity. Alkynes combining electronic and steric effects show a general tendency to place the bulkier group away from the metal center. The predictability of these reactions allows for the incorporation of either photoactive (anthracene or pyrene) or electroactive (ferrocene) labels in the nucleotide or nucleoside moiety. The detection of these labels can be easily achieved by UV/Vis spectroscopy or electrochemistry, as has been demonstrated by comparison with the parent (unlabeled) compounds.

The methodology reported could have applications as a site-specific labeling technique for oligonucleotides with easily detectable orthogonal markers. The strategy, based on the selective postfunctionalization of oligonucleotides (metalation of specific modified bases within the chain and subsequent incorporation of the label), differs from those reported for modified DNA fragments that rely on the assembly of labeled units. Translation of these results to the functionalization of oligonucleotides or arylpurine/pyrimidine-containing DNA fragments will be attempted in the near future in our laboratories.

Acknowledgements

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Keywords: iridium · labeling · metallacycles · nucleobases · rhodium

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